

# UC San Diego

## UC San Diego Previously Published Works

### Title

A role for kinesin heavy chain in controlling vesicle transport into dendrites in *Drosophila*.

### Permalink

<https://escholarship.org/uc/item/4fd2d1qk>

### Journal

Molecular biology of the cell, 22(21)

### ISSN

1059-1524

### Authors

Henthorn, Kristina Schimmelpfeng  
Roux, Meike Sabina  
Herrera, Cheryl  
et al.

### Publication Date

2011-11-01

### DOI

10.1091/mbc.e10-07-0572

Peer reviewed

# A role for kinesin heavy chain in controlling vesicle transport into dendrites in *Drosophila*

Kristina Schimmelpfeng Henthorn<sup>a</sup>, Meike Sabina Roux<sup>b</sup>, Cheryl Herrera<sup>a</sup>, and Lawrence S. B. Goldstein<sup>a</sup>

<sup>a</sup>Howard Hughes Medical Institute and Department of Cellular and Molecular Medicine, University of California, San Diego, School of Medicine, La Jolla, CA 92093-0683; <sup>b</sup>Department for Neuroanatomy and Molecular Brain Research, Ruhr-Universität Bochum, D-44780 Bochum, Germany

**ABSTRACT** The unique architecture of neurons requires the establishment and maintenance of polarity, which relies in part on microtubule-based transport to deliver essential cargo into dendrites. To test different models of differential motor protein regulation and to understand how different compartments in neurons are supplied with necessary functional proteins, we studied mechanisms of dendritic transport, using *Drosophila* as a model system. Our data suggest that dendritic targeting systems in *Drosophila* and mammals are evolutionarily conserved, since mammalian cargoes are moved into appropriate domains in *Drosophila*. In a genetic screen for mutants that mislocalize the dendritic marker human transferrin receptor (hTfR), we found that kinesin heavy chain (KHC) may function as a dendritic motor. Our analysis of dendritic and axonal phenotypes of KHC loss-of-function clones revealed a role for KHC in maintaining polarity of neurons, as well as ensuring proper axonal outgrowth. In addition we identified adenomatous polyposis coli 1 (APC1) as an interaction partner of KHC in controlling directed transport and modulating kinesin function in neurons.

## Monitoring Editor

Xueliang Zhu  
Chinese Academy of Sciences

Received: Jul 7, 2010

Revised: Jul 11, 2011

Accepted: Aug 25, 2011

## INTRODUCTION

Neurons are highly polarized cells that contain two spatially and functionally distinct domains, the axonal and the somatodendritic domains. These domains maintain unique membrane structures and protein compositions and are essential for the neuron to receive and transmit signals. Disruption of this cellular organization can lead to loss of activity and neuronal death. While recent research sheds light on the mechanisms that control establishment of neuronal polarity during development and the involvement of microtubule-based motors, little is known about motor protein function and regulation during maintenance of polarity in differentiated neurons (Hirokawa and Takemura, 2005; Arimura and Kaibuchi, 2007).

Studies in cultured hippocampal neurons suggest a role of selective microtubule-based transport in the polarized distribution of at least a subset of dendritic proteins (Burack *et al.*, 2000; Silverman *et al.*, 2001). This seems to be a common mechanism for the localization of dendritic proteins, although the generality of this process remains to be proven. It has been suggested that so-called “smart” motor proteins that can distinguish between different subcellular destinations might mediate selective transport into dendrites (Burack *et al.*, 2000; Goldstein and Yang, 2000; Shah and Goldstein, 2000). Three different hypotheses could explain how a smart motor can generate directed movement into dendrites. In the “structural hypothesis,” a smart motor could read structural differences between the axonal and dendritic cytoskeletons, such as composition of tubulin variants or posttranslational modifications (e.g., phosphorylation, acetylation or decoration with different microtubule-associated proteins [tau, MAP2]). Alternatively, in the “regulatory hypothesis,” a smart motor could be differentially regulated by the environment or cargo it transports. Finally, in the “mixed polarity” hypothesis, maintenance of polarity could be achieved by the selective use of minus-end motor proteins that cannot move into axons where plus-end microtubules are pointing away from the cell body, but can move into dendrites that contain mixed polarity microtubules that point either their plus or their minus ends away from the cell body (Baas *et al.*, 1988).

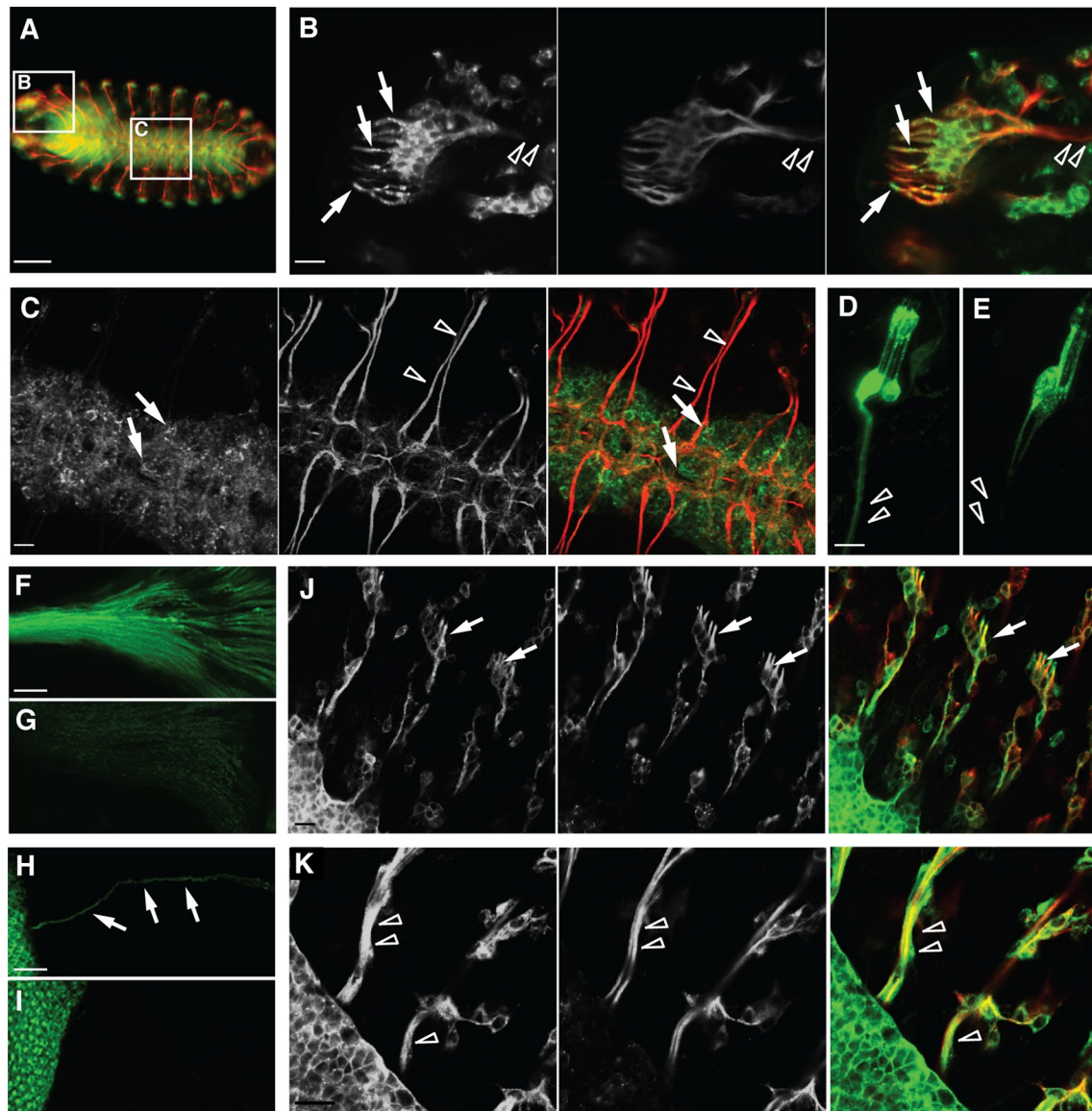
This article was published online ahead of print in MBoC in Press (<http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E10-07-0572>) on August 31, 2011.

Address correspondence to: Lawrence S. B. Goldstein ([lgoldstein@ucsd.edu](mailto:lgoldstein@ucsd.edu)).

Abbreviations used: APC, adenomatous polyposis coli; CSP, cysteine string protein; GFP, green fluorescent protein; hTfR, human transferrin receptor; MARCM, mosaic analysis with a repressible cell marker; PBS, phosphate-buffered saline; PNS, peripheral nervous system.

© 2011 Henthorn *et al.* This article is distributed by The American Society for Cell Biology under license from the author(s). Two months after publication it is available to the public under an Attribution–Noncommercial–Share Alike 3.0 Unported Creative Commons License (<http://creativecommons.org/licenses/by-nc-sa/3.0>).

“ASCB,” “The American Society for Cell Biology,” and “Molecular Biology of the Cell” are registered trademarks of The American Society of Cell Biology.



**FIGURE 1:** hTfR-GFP localizes to dendrites in *Drosophila* neurons. (A–C, E, G, I, and J) elav-Gal4 x UAS-hTfR-GFP. (D, F, H) elav-Gal4 x UAS-cd8-GFP. (A–C) Ventral view of stage 17 embryo, anti-GFP (green) and 22C10 (red). (B) Chordotonal organs of the head. (C) Ventral nerve cord. hTfR localizes to cell bodies and dendrites (arrows) but fails to enter distal axons (arrowheads). (D and E) hTfR-GFP does not enter distal axons in 3rd instar chordotonal organs (E, arrowheads) compared with cd8-GFP (D, arrowheads) and is largely excluded from photoreceptor axons (G) and Bolwig's nerve (I) in 3rd instar larvae compared with cd8-GFP (F and H, arrows). (J and K) elav-Gal4 x UAS-ΔC-hTfR-GFP. Deletion of the cytoplasmic domain results in dendritic (arrows), as well as axonal, localization (arrowheads). Scale bar: 50 μm (A); 10 μm (B–E), (J–K); 25 μm (F–I).

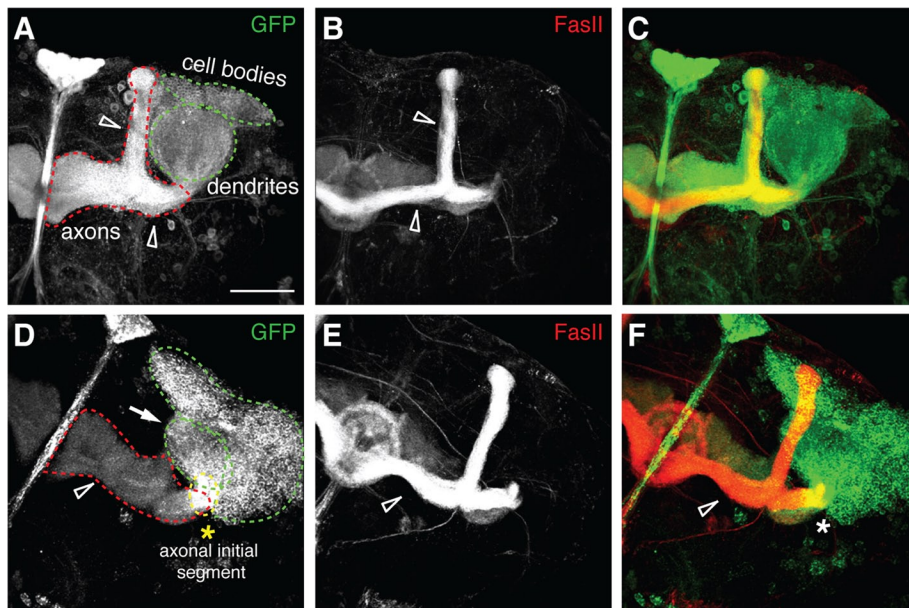
We took an *in vivo* genetic approach to test these hypotheses for polarized protein transport in neurons. We examined the subcellular localization of the dendritic protein human transferrin receptor (hTfR) in *Drosophila* neurons and subsequently searched for mutants that interfere with its localization.

## RESULTS

### Human transferrin receptor localizes preferentially to dendrites in *Drosophila* neurons

A well-studied protein that localizes to dendrites in mouse hippocampal neurons via selective transport is hTfR (Burack *et al.*, 2000). To explore whether this localization mechanism is evolutionarily conserved, we took advantage of the Gal4 system (Brand and Perrimon, 1993) and compared the subcellular ex-

pression pattern of a green fluorescent protein (GFP)-tagged version of hTfR in *Drosophila* neurons to the expression of cd8-GFP. Because cd8 is a transmembrane glycoprotein, its expression allows visualization of all cellular extensions (Lee and Luo, 1999). As in vertebrates, hTfR-GFP vesicles localize preferentially to dendrites in all neuronal cell types that we studied. GFP-positive vesicles can be found in dendrites but not distal axons in embryonic peripheral nervous system (PNS) and CNS neurons, compared with MAb 22C10, which stains all cellular processes in all PNS and some CNS neurons (Fujita *et al.*, 1982; Figure 1, A–C). In 3rd instar larvae, hTfR-GFP vesicles are present in dendrites but fail to enter axons beyond the initial segment in PNS neurons (Figure 1E, arrowheads). In comparison, cd8-GFP can be found throughout the cell (Figure 1D, arrowheads). In addition,



**FIGURE 2:** Differential distribution of hTfR-GFP and kif21a-GFP in adult mushroom bodies. GFP-fusion proteins are expressed using the mushroom body-specific OK107Gal4 driver. (A–C) OK107Gal4, kif21a-EGFP stained with anti-GFP (green) and anti-FasII (red). The axonal kinesin kif21a is enriched in axons in adult mushroom bodies and overlaps with the axonal cell adhesion molecule fasII (arrowheads). (D–F) OK107Gal4, hTfR-GFP stained with anti-GFP (green) and anti-FasII (red). hTfR-GFP is enriched in cell bodies and dendrites in adult mushroom bodies and does not colocalize with FasII staining in distal axons. However, note the strong localization of hTfR-GFP to the axonal initial segment (asterisk). Scale bar: 50µm.

hTfR-GFP is largely excluded from photoreceptor axons (Figure 1G) and Bolwig's nerve (Figure 1I), compared with cd8-GFP (Figure 1H, arrows).

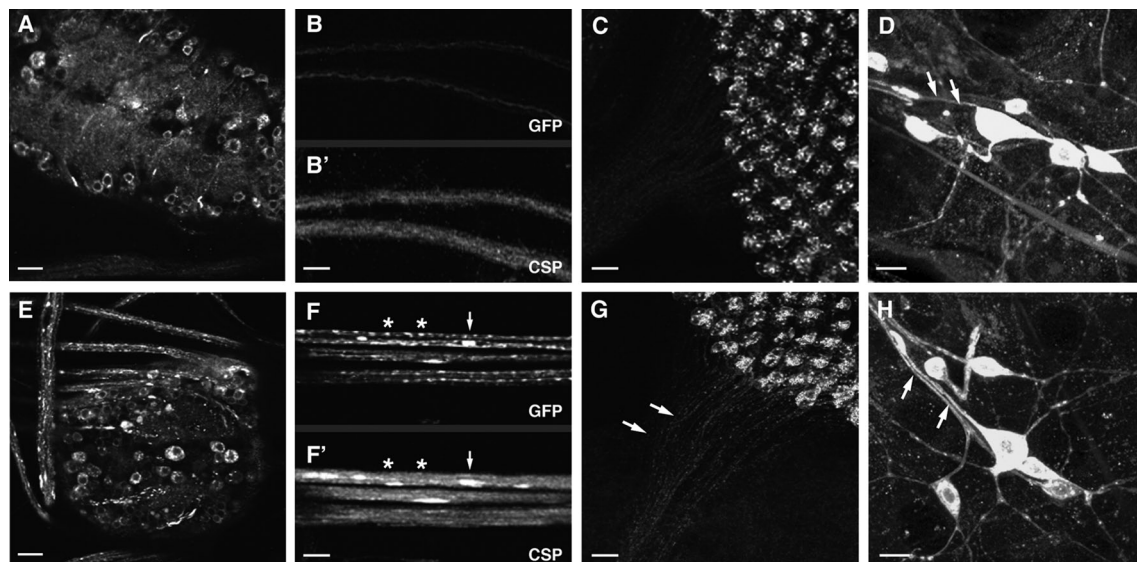
Interestingly, when we expressed a hTfR-GFP construct that lacks the cytoplasmic domain, we observed strong localization to axons (Figure 1K, arrowheads) and dendrites (Figure 1J, arrows), similar to what was previously described in vertebrate tissue culture (West et al., 1997). In addition, the anti-GFP staining reveals a stronger localization of GFP to neurite membranes, as opposed to vesicular staining, which confirms the finding that the cytoplasmic domain of hTfR contains the dendritic targeting signal and the internalization signal (West et al., 1997).

We then compared hTfR localization to the subcellular distribution of an axonal marker, the kinesin family member kif21a (Marszalek et al., 1999). kif21a-GFP strongly localizes to axons in adult mushroom bodies (Figure 2, A–C), in contrast to hTfR-GFP, which is found in cell bodies, dendrites, and the axonal initial segment of mushroom-body neurons, while being excluded from distal axons (Figure 2, D–E).

### Mutations in the kinesin heavy chain gene mislocalize hTfR-GFP to axons

To identify genes required for dendritic localization, we performed a loss-of-function screen to identify mutants in which hTfR-GFP mislocalizes to peripheral nerve axons in 3rd instar larvae.

Initially, we screened a collection of 45 mutants in heterozygous condition for axonal appearance of hTfR-GFP in optic nerves and



**FIGURE 3:** Mutations in the kinesin heavy chain gene lead to mislocalization of hTfR-GFP to axons. In +/+, elavGal4/UAS-hTfR-GFP larvae, hTfR-GFP fails to enter segmental nerves (B) and CSP staining appears smooth (B'). (E–H) *khc8/khc1<sup>ts</sup>*; elavGal4/UAS-hTfR-GFP larvae. At 29°C, hTfR-GFP enters segmental nerves and forms organelle accumulations (F). Some of the GFP-positive clogs overlap with CSP-positive organelle accumulations (F, F', arrow), others don't (F, F', asterisk). In addition, a mislocalization into optic nerves can be observed in the *Khc* mutants (G, arrows). (A and E) hTfR-GFP expression level in cell bodies of the ventral ganglion is comparable. In multidendritic cells of 3rd instar *khc8/khc1<sup>ts</sup>* larvae, a mislocalization of GFP into axons is evident (H, arrows) compared with wild type (D, arrows).



Structural proteins	Signaling/scaffolding	Motors
gammaTub37C	Dsor	dhc
alphaTub84B	ksr	robl
betaTub60D	chrw	grid
alphaTub67C	phl	khc
betaTub85D	Ras85D	Klp 64D
futsch K68	rl (Df)	KLP61F
futsch N94	puc	KLP31E (Df)
futsch P28	bsk	nod
UAS-Map2	syd	ncd
UAS-Tau	msn	neb
Eb1	Ptp10D	pav
cnr	Ptp69D	
stau	14-3-3zeta	
	UAS-cdc42V12	
	Gβ	
	Gγ	
	R(Rap1)	
	Rab5	
	Rab6	
	Rab8 (Df)	
	Rab11	
	Abl/TM6B	
	aPKC	
	Rho1	
	par-1	
	pka-C1	
	polo	
	AP-47 (Df)	
	ebi	
	shi	

**TABLE 1:** Candidate mutants screened for mislocalization of hTfR-GFP to axons in 3rd instar larvae.

segmental nerves as candidates to test the structural hypothesis, the regulatory hypothesis, and the mixed polarity model (Table 1). None of the genes tested gave strong phenotypes under these conditions.

However, when we examined more carefully, we found that mutations in *kinesin heavy chain* (*khc*), the force-generating subunit of *Drosophila kinesin-I* (Saxton et al., 1991), led to axonal accumulation of hTfR-GFP-positive vesicles. To overcome early larval lethality associated with *khc* loss-of-function alleles, we analyzed transheterozygote *khc<sup>8</sup>/khc<sup>1ts</sup>* animals. When raised at the restrictive temperature of 29°C, *khc<sup>8</sup>/khc<sup>1ts</sup>* larvae survive to 3rd instar larval stages but exhibit a strong tail-flipping phenotype and accumulation of synaptic vesicle markers in segmental nerves (Saxton et al., 1991; Hurd and Saxton, 1996).

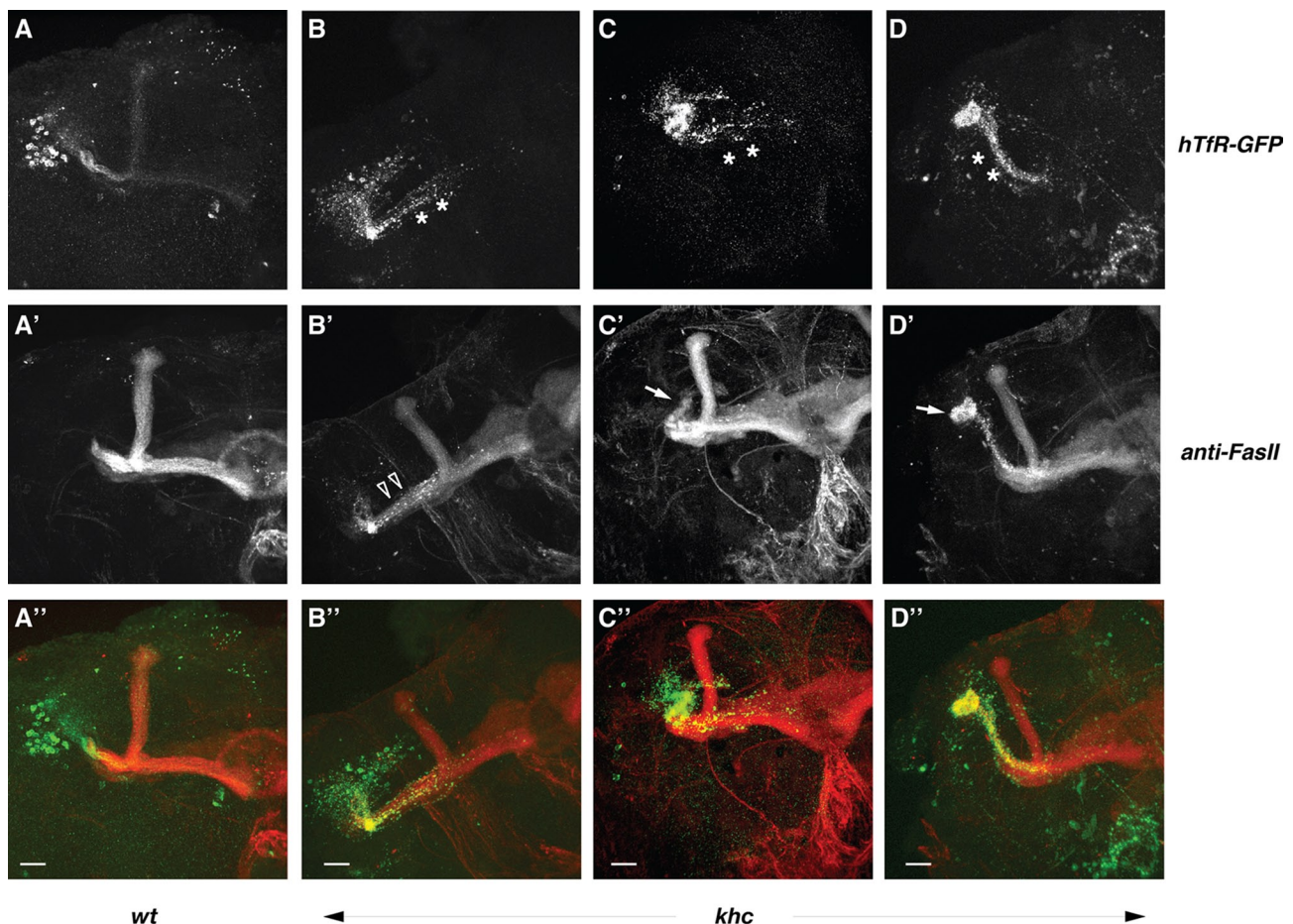
We observed obvious localization of hTfR-GFP in axons of segmental nerves, optic nerves, and multidendritic cells of *khc<sup>8</sup>/khc<sup>1ts</sup>* larvae (Figure 3, F–H). This aberrant localization is not caused by

elevated expression levels of hTfR-GFP in mutants, since similar fluorescence intensity is observed in *khc<sup>+</sup>/khc<sup>+</sup>* and *khc<sup>8</sup>/khc<sup>1ts</sup>* mutant animals (Figure 3, A and E). No significant mislocalization into axons of hTfR-GFP could be observed in animals heterozygous for *khc<sup>8</sup>* (unpublished data).

We next tested whether complete loss of *khc* function affects the localization of other proteins in addition to hTfR-GFP. To exclude residual maternal contributions of Khc and to express hTfR-GFP in mutant cells, we generated loss-of-function neuroblast clones in mushroom bodies using the mosaic analysis with a repressible cell marker (MARCM) technique (Lee and Luo, 1999). In wild-type clones, the distribution of hTfR-GFP was as expected, with strong accumulation in the axonal initial segment and most GFP vesicles being found in cell bodies and dendrites (Figure 4A). To label axons in mushroom bodies, we used mAb1D4 (anti-FasII). FasII immunoreactivity can be found in the axons of  $\alpha$  and  $\beta$  lobes, and at reduced levels in the  $\gamma$  lobes. FasII is not ordinarily expressed in wild-type cell bodies and the calyx (dendrites; Figure 4A'; Crittenden et al., 1998). In *khc* clones, a range of phenotypes was observed. As expected, hTfR-GFP strongly mislocalized to and accumulated in axons beyond the initial segment (Figure 4, B–D). Severe neuronal morphogenetic defects were also uncovered using the FasII marker. Abnormal FasII accumulations were found in axons and pathfinding, and fasciculation and branching was disrupted, resulting in extra-long lobes, thinner lobes, or additional FasII-positive branches (Figure 4, B'–D'). In addition, we found mislocalization of FasII to the calyx and cell bodies (Figure 4D'). While the mislocalization of hTfR-GFP vesicles could be observed in all clones analyzed, the severity of the neuron morphogenetic phenotypes was more variable. We found different expression of fasciculation defects in 90% of the clones, but a clear ectopic expression of FasII specifically was observed in only 20% of the clones. Together, these phenotypes point toward a role of Khc in delivering guidance receptors, cell adhesion molecules, and other cargoes to their appropriate subcellular sites during development.

#### Identification of APC1 as a potential *khc*-dependent dendritic localization factor in cytological location 98E3-F5

Our data suggest that Khc is required to transport hTfR-GFP into dendrites. We next asked what might regulate directionality of Khc movement. To set up a modifier screen, we took advantage of the fact that a 50% reduction in Khc does not lead to mislocalization of the hTfR-GFP marker, whereas a more pronounced protein reduction in the *khc<sup>8</sup>/khc<sup>1ts</sup>* animals results in axonal entry of hTfR-GFP. After screening 50 third-chromosomal deficiencies for enhancers of Khc, we recovered eight lines that, when transheterozygous with *khc<sup>8</sup>*, led to considerable hTfR-GFP appearance in optic nerves (Table 2). Among these, the strongest mislocalization was observed in Df(3R)3450 (Figure 5B). Since Df(3R)3450 spans the cytological location 98E3-99A6, we could use additional deficiencies to narrow down the region that contains the Khc enhancer to cytological location 98E3-F5 (unpublished data). Among the 34 annotated genes in this region (Wilson et al., 2008), we found Apc1 to be an interesting candidate for a Khc interaction partner, because *Drosophila* APC1 is highly enriched in axons throughout development (Hayashi et al., 1997; Ahmed et al., 1998). In addition, APC proteins could bind microtubules (Munemitsu et al., 1994; Smith et al., 1994 [Drosophila APC1 contains a potential microtubule-binding domain; Hayashi et al., 1997; Goldstein and Gunawardena, 2000]), and APC may form a complex with mPar3 and Kif3A to initiate axon specification in developing hippocampal neurons (Shi et al., 2004).



**FIGURE 4:** Khc established proper neuronal morphology during development. (A) wild-type mushroom-body clones. hTfR-GFP localizes to cell bodies, dendrites, and the axonal initial segment (A), anti-FasII staining in axons (A'). (B–D) *khc8/khc8* mushroom-body clones. hTfR-GFP is strongly mislocalized to axons beyond the initial segment (asterisks). EGFP-positive organelle accumulations can be observed. Other phenotypes include elongated axonal projections visible with both hTfR-GFP (B) and anti-FasII (B' arrowheads), ectopic axonal branching (C, C', arrow), and mislocalization of FasII to cell bodies and thinner axonal lobes (D, D', arrow). Scale bar: 20 μm.

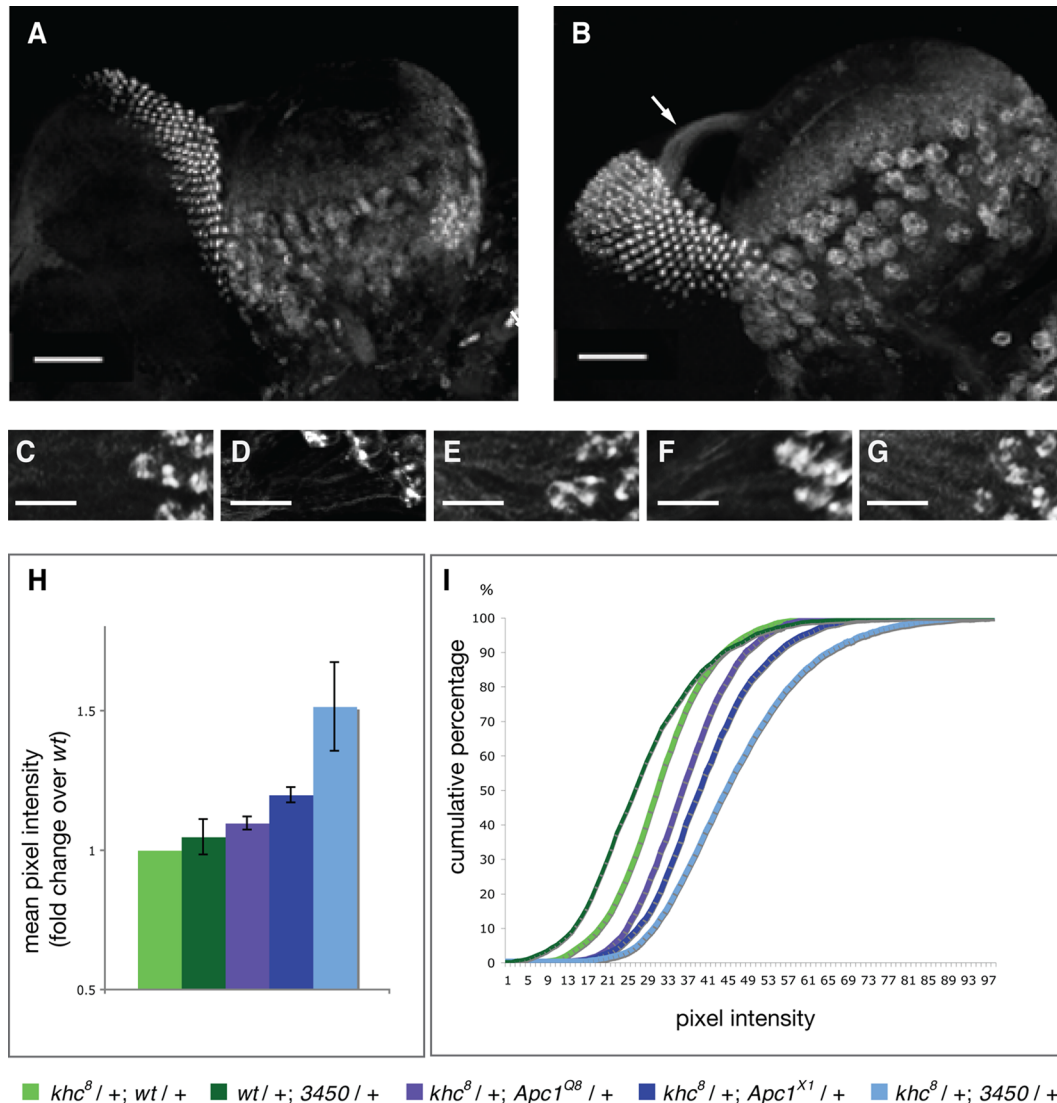
To quantify localization, we measured the mean fluorescence intensity per pixel of hTfR-GFP in optic nerves of 3rd instar larvae in different *khc8/+*; *apc1/+* transheterozygote backgrounds and com-

pared them with a simple *khc8* /+ heterozygote or *Df(3R)3450/+* heterozygote. (Figure 5, C–G). We found a 1.36-fold increase in mean pixel intensity in *khc8/+*; *Df(3R)3450/+* transheterozygote animals over *khc8/+*; *wt/+* and a 1.1- to 1.22-fold increase with two different *apc1* alleles (*khc8/+*; *apc1<sup>Q8</sup>/+* and *khc8/+*; *apc1<sup>1</sup>/+*; Figure 5H). In addition, when we calculated the cumulative pixel intensity distribution, we observed a statistically significant shift to higher pixel intensities in the *khc8/+*; *apc1/+* transheterozygotes (Figure 5I). Thus the reduction of Apc1 enhances the phenotype of *khc* reduction and Apc1 and Khc may interact to control the transport of hTfR into dendrites.

Homozygous *apc1<sup>Q8</sup>* animals survive to the 3rd instar larval stage at the restrictive temperature of 29°C. We therefore investigated whether we could observe a mislocalization of hTfR-GFP into axons in homozygous animals with wild-type amounts of Khc. Clear mislocalization of hTfR-GFP in optic nerves was found in homozygous *apc1<sup>Q8</sup>* larvae compared with wt (Figure 6B). Quantification of mean pixel intensity in optic nerves revealed a 1.4-fold increase in *apc1<sup>Q8</sup>* over wt (Figure 6C). In addition, since Apc1 is highly abundant in axons, we asked whether we could observe a transport defect in *apc1* homozygous animals. We stained segmental nerves with cysteine string protein (CSP) antibodies (Zinsmaier et al., 1994) and observed accumulation of this synaptic vesicle marker in the nerves

Degree mislocalization	Bloomington number	Name	Breakpoints
+++	430	Df(3R)3450	98E3; 99A6
++	6754	Df(3L)fz2	75F10-11; 76A1-5
++	6646	Df(3L)BSC20	76A7-B1; 76B4-5
++	5126	Df(3L)XS533	76B4; 77B1
++	5694	Df(3R)e1025-14	82F8-10; 83A1-3
+	3124	Df(3L)fz-GF3b	70C2; 70D5
+	3546	Df(3R)B81	99D3-3Rt
+	8103	Df(3R)ED5177	83B4; 83B6

**TABLE 2:** Third chromosomal deficiencies that lead to mislocalization of hTfR-GFP in a *khc8/+* background.



**FIGURE 5:** Identification of APC1 as a potential Khc-dependent dendritic localization factor. (A) UAS-hTfR-GFP, *khc8/+*; *elavGal4/+*, (B) UAS-hTfR-GFP, *khc8/+*; *elavGal4/ Df(3R)3450*. Note the strong mislocalization of GFP to the optic nerve (B, arrow). Scale bar: 20  $\mu$ m. (C) UAS-hTfR-GFP, *khc8/+*; *elavGal4/+*, (D) UAS-hTfR-GFP, *+/+*; *elavGal4/ Df(3R)3450*, (E) UAS-hTfR-GFP, *khc8/+*; *elavGal4/ apc1<sup>Q8</sup>*, (F) UAS-hTfR-GFP, *khc8/+*; *elavGal4/ apc1<sup>X1</sup>*, (G) UAS-hTfR-GFP, *khc8/+*; *elavGal4/ Df(3R)3450*. Examples of optic nerves used for the quantification. Scale bar: 10  $\mu$ m. (H) Note the increase in hTfR-GFP mean pixel intensity in optic nerves of the *khc8*; APC1 transheterozygotes compared with the *khc8* heterozygotes. (I) The statistically significant left shift in the curves indicates a higher percentage of bright pixels in optic nerves of the *khc8*; APC1 transheterozygote animals compared with the *khc8* heterozygotes (*khc8*; *Df(3R)3450*  $\rightarrow$  *p* = 8.3405e-005; *X1*  $\rightarrow$  *p* = 2.1768e-005; *Q8*  $\rightarrow$  *p* = 0.0301, *p* values were obtained by running a Kolmogorow-Smirnov test).

of *apc1<sup>Q8</sup>* larvae (Figure 6E). We quantified this phenotype and counted 2.47 clogs/mm nerve compared with 0.09 clogs/mm nerve in *yw* control larvae (Figure 6F).

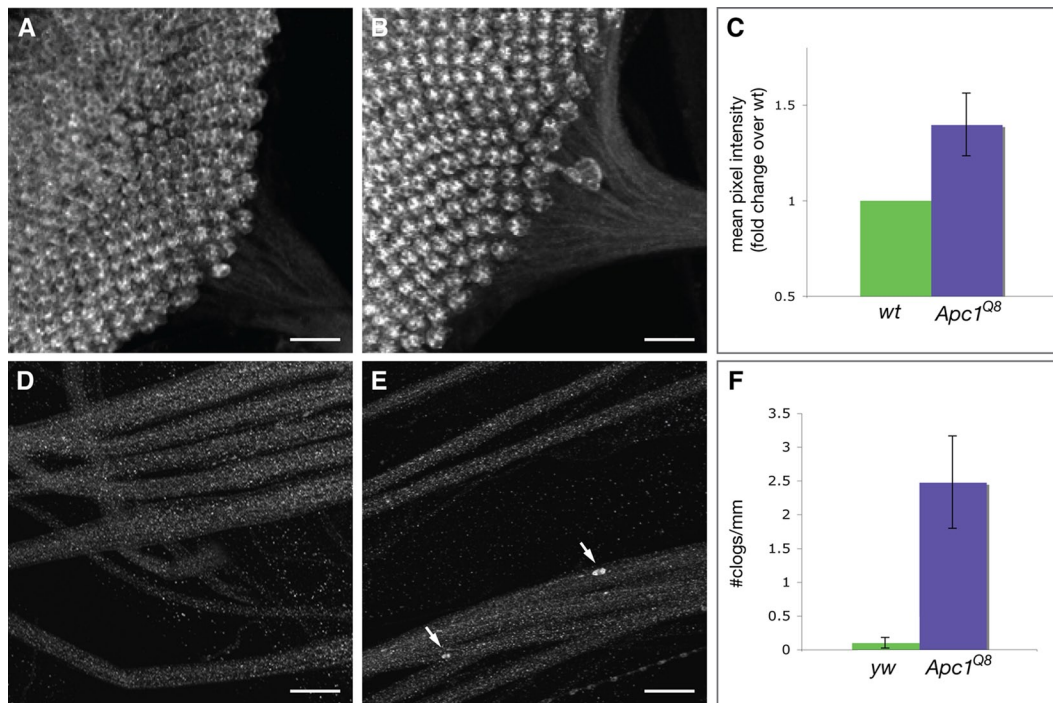
## DISCUSSION

In this study, we evaluated the involvement of microtubule-based transport in maintaining neuronal polarity and identified Khc as a motor that can mediate transport of cargo into dendrites. We established a model system in *Drosophila* by expressing a vesicular protein that is actively transported into dendrites, hTfR coupled to GFP (Burack *et al.*, 2000). We found that the subcellular localization in dendrites of this vertebrate protein was mirrored in invertebrate neurons (Figure 1), which indicates the mechanisms that mediate its

polarized transport are evolutionarily conserved. Using hTfR-GFP, we were able to take advantage of *Drosophila* genetics to test for the existence of a smart motor.

In our loss-of-function screen for mutants that mislocalize the dendritic hTfR-GFP construct to axons, we identified a *khc* mutant that led to strong axonal accumulations of the hTfR-GFP. Interestingly, when we analyzed mutant *khc* clones in mushroom bodies, we observed a variety of neuron morphogenetic phenotypes, such as axonal pathfinding failures as well as defects in fasciculation and branching, in addition to the mislocalization of hTfR-GFP (Figure 4). Therefore, in addition to previously described roles for Khc in establishing the anterior–posterior axis in the oocyte and axonal transport of synaptic vesicles (Hurd and Saxton, 1996; Brendza *et al.*, 2000),





**FIGURE 6:** APC1 functions in transport of dendritic as well as axonal vesicles. (A and D) wt and (B and E) *apc1<sup>Q8</sup>/apc1<sup>Q8</sup>*. A mislocalization of hTfR-GFP can be observed in the mutants compared with wt (B), as well as the appearance of CSP-positive organelle accumulations in segmental nerves (E, arrows). (C) Quantification of hTfR-GFP mean pixel intensity. (F) Quantification of CSP clogs.

our data show that the kinesin motor is also needed throughout development to transport important guidance molecules to the growth cone and to carry dendritic cargo to its destination.

Khc is a plus end-directed motor that moves toward the plus ends of microtubules, which in axons are located toward the synapse. In vertebrates, dendrites have been shown to contain microtubules with mixed polarity (Baas *et al.*, 1988), thus allowing plus-end motors such as kinesin to enter dendrites and axons. A recent study showed that proximal dendrites are equipped with 90% minus end-out microtubules in some *Drosophila* neurons (Stone *et al.*, 2008), which would make kinesin an unlikely candidate to transport cargo into dendrites. However, in our study of *khc* loss-of-function phenotypes in segmental nerves, optic nerves, and mushroom bodies, we could show a mislocalization of the dendritic hTfR to axons (Figures 3 and 4). This clearly indicates that hTfR is a Khc cargo and suggests that Khc is able to actively transport hTfR into dendrites, despite a scarcity of plus end-out microtubules. Interestingly, we failed to observe an axonal mislocalization of hTfR-GFP in mutants of the minus end-directed dynein or dynactin motors (unpublished data), further supporting our model of hTfR-GFP being transported by a plus-end motor.

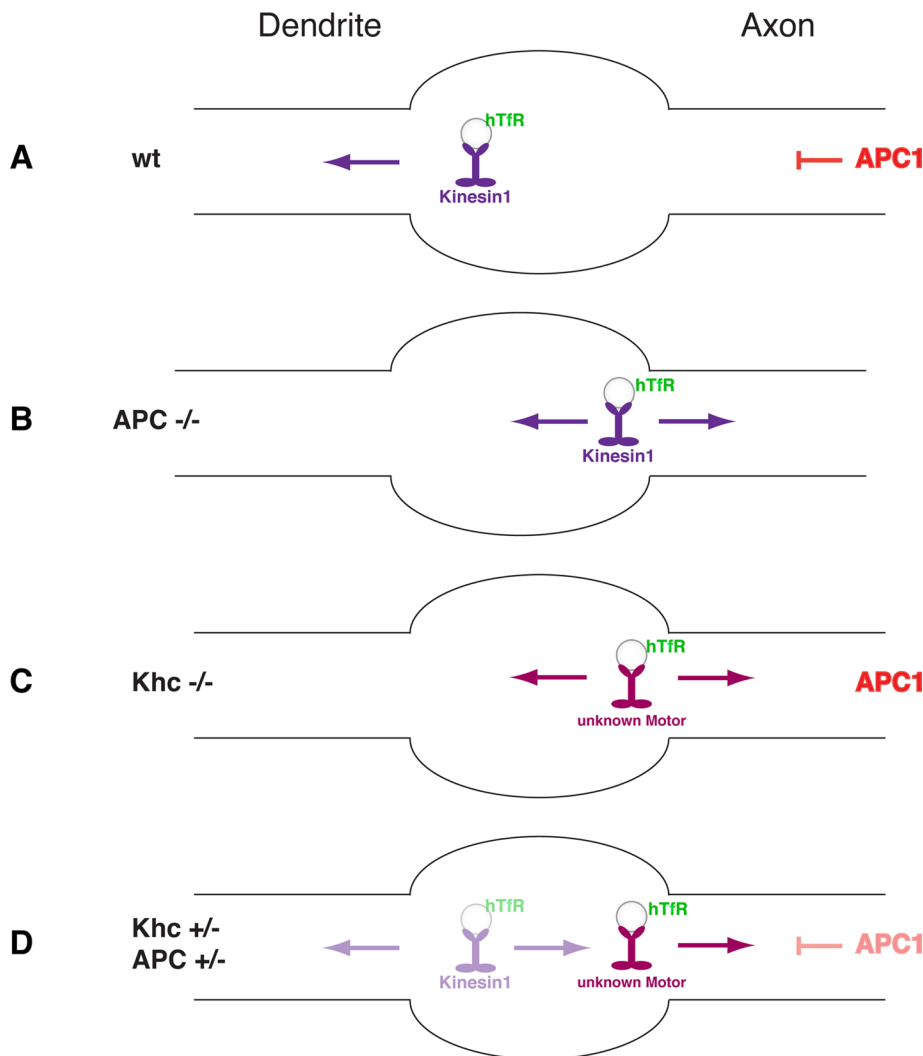
We suggest that Khc can be classified as a smart motor that actively travels in both axonal and dendritic compartments and, depending on its localization and/or cargo, must be differentially regulated. Alternatively, Khc could be part of a mechanism that excludes some dendritic proteins from falsely entering the axon. If Khc is able to enter various compartments and transports axonal as well as dendritic cargoes, how is its direction of travel determined? In a secondary modifier screen, we aimed to identify interaction partners of kinesin that control its localization and/or direction of travel. We found *apc1*, the *Drosophila* homologue of the tumor suppressor adenomatous polyposis coli (APC; Hayashi *et al.*, 1997) to be a neuronal Khc

modifier. Since d-APC or APC1 is a microtubule-binding protein and highly expressed in axons during development (Munemitsu *et al.*, 1994; Smith *et al.*, 1994; Hayashi *et al.*, 1997; Ahmed *et al.*, 1998), it was conceivable that it could interact with Khc in axons to prevent dendritic cargoes from being transported. In addition, it has been reported that APC colocalizes with Khc in epithelial cells and that disruption of Khc function abolishes peripheral APC localization (Cui *et al.*, 2002), further supporting our finding of a possible collaboration between these two proteins in controlling directional transport.

Indeed, when we measured axonal appearance of hTfR-GFP in transheterozygote *khc/apc1* animals, we found a significant increase in mislocalization of hTfR-GFP in optic nerve axons with several *apc1* alleles and the deficiency when compared with *khc<sup>8</sup>* heterozygote animals alone. Therefore APC1 cooperates with KHC in mediating dendritic restriction of hTfR. In addition, APC1 homozygous larvae that survive until the 3rd instar stages also show mislocalization of hTfR-GFP into axons, as well as a pronounced accumulation of synaptic vesicles in segmental nerves, thus revealing a previously unknown function for APC1 in transport in differentiated neurons. In developing hippocampal neurons, a similar role for vertebrate APC has been reported (Shi *et al.*, 2004). In that study, the authors show that the polarity marker mPar3 is localized to the tip of growing axons via APC and kinesin-2-mediated transport and spatially regulated GSK-3 $\beta$ , confirming the tendency of APC proteins to associate with plus end-directed motors.

In summary, our data and the data of others reveal that mutations in both Khc and APC1 genes lead to defects in the localization of axonal and dendritic cargoes in differentiated *Drosophila* neurons. The data presented here allow us to propose the following model: in the wild-type neuron, axonally located APC1 prevents kinesin-1-bound hTfR from entering (Figure 7A). Therefore, in *apc1*





**FIGURE 7:** A model of Khc function in dendritic transport. (A) Wild-type neuron, (B) APC mutant neuron, (C) Khc mutant neuron, and (D) APC/Khc transheterozygote neuron.

homozygous mutants, kinesin-I aberrantly transports hTfR into axons (Figure 7B). In *khc* homozygous mutants, an unknown, generic motor protein binds to the dendritic cargo and randomly transports it into all compartments (Figure 7C). Similarly, in the transheterozygote *khc/apc1* animals, axonal exclusion is no longer strong enough to prevent some dendritic hTfR bound to Khc from localizing into axons, and the decrease of a preferred motor allows some hTfR to bind to an unknown, generic motor (Figure 7D).

Taken together, our data strongly support the existence of a dynamic, smart motor that, dependent on its environment and cargo, can control the establishment and maintenance of neuronal polarity, thereby ensuring proper nervous system function.

## MATERIALS AND METHODS

### *Drosophila* stocks and genetics

Flies were raised on conventional medium at 25°C, and all fly work and recombination experiments were done according to common practice. *apc1* stocks were obtained from Eric Wieschaus (Princeton University, Princeton, NJ). All other stocks were obtained from the Bloomington stock center (Bloomington, IN). The hTfR-GFP construct, a kind gift from M. Silverman and Gary Banker (Oregon Health Sciences University, Portland, OR), was subcloned into the

pUAST vector, and transgenic flies were generated by P-element-mediated germline transformation.

Genetic mosaics were generated using the MARCM system (Lee and Luo, 1999) with FRT40, tub-Gal80 on the second chromosome and a elav-Gal 4 driver on the third chromosome. To induce mitotic recombination in mushroom-body neuroblasts, staged larvae were heat-shocked for 30–40 min in a 37°C water bath 0–4 h after larval hatching, and then returned to 25°C until analysis.

### Immunostaining

Larval segmental nerve immunostaining was performed as described previously (Hurd and Saxton, 1996). Immunostaining of embryos was done as described previously (Hummel et al., 1997). Immunostaining of wandering 3rd instar larval or adult nervous systems was done as follows: brains with attached segmental nerves and eye-antenna imaginal discs were dissected in phosphate-buffered saline (PBS; pH 7.2) and fixed with 4% formaldehyde for 20 min at room temperature. The samples were then rinsed and washed three times for 10–15 min each time in PBT (PBS, 0.3% Triton X-100) and blocked for 1 h in PBT plus 10% goat serum. Primary antibodies were incubated overnight at 4°C. The next day, samples were washed and incubated in secondary antibodies for 2 h at room temperature, washed again, and mounted in Vectashield (Vector Laboratories, Burlingame, CA).

The following antibodies were obtained from the Developmental Studies Hybridoma Bank at the University of Iowa: 22C10 (Fujita et al., 1982), anti-FasII: 1D4 (Goodman, unpublished data), and CSP (Zinsmaier et al., 1994). Anti-GFP (rabbit serum; Molecular Probes, Carlsbad, CA), goat anti-mouse and goat anti-rabbit Alexa Fluor 488- and Alexa Fluor 594-coupled secondary antibodies were from Molecular Probes. All images were taken on an Olympus FV1000 spectral deconvolution confocal microscope (Olympus, Center Valley, PA) and processed using Image J (National Institutes of Health).

### ACKNOWLEDGMENTS

We thank Lukasz Szpankowski for help with statistical analyses and the University of California, San Diego, Neuroscience Microscopy Shared Facility Grant P30 NS047101 team, especially Brendan Brinkman and Jennifer Meerloo. K.S.H. was supported by the Deutsche Forschungsgemeinschaft (DFG SCHI 598/3-1). L.S.B.G. is an investigator of the Howard Hughes Medical Institute.

### REFERENCES

- Ahmed Y, Hayashi S, Levine A, Wieschaus E (1998). Regulation of armadillo by a *Drosophila* APC inhibits neuronal apoptosis during retinal development. *Cell* 93, 1171–1182.
- Arimura N, Kaibuchi K (2007). Neuronal polarity: from extracellular signals to intracellular mechanisms. *Nat Rev Neurosci* 8, 194–205.

- Baas PW, Ditch JS, Black MM, Banker GA (1988). Polarity orientation of microtubules in hippocampal neurons: uniformity in the axon and nonuniformity in the dendrite. *Proc Natl Acad Sci USA* 85, 8335–8339.
- Brand AH, Perrimon N (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118, 401–415.
- Brendza RP, Serbus LR, Duffy JB, Saxton WM (2000). A function for kinesin I in the posterior transport of oskar mRNA and Staufen protein. *Science* 289, 2120–2122.
- Burack MA, Silverman MA, Banker G (2000). The role of selective transport in neuronal protein sorting. *Neuron* 26, 465–472.
- Crittenden JR, Skoulakis EM, Han KA, Kalderon D, Davis RL (1998). Tripartite mushroom body architecture revealed by antigenic markers. *Learn Mem* 5, 38–51.
- Cui H, Dong M, Sadhu DN, Rosenberg DW (2002). Suppression of kinesin expression disrupts adenomatous polyposis coli (APC) localization and affects  $\beta$ -catenin turnover in young adult mouse colon (YAMC) epithelial cells. *Exp Cell Res* 280, 12–23.
- Fujita SC, Zipursky SL, Benzer S, Ferrus A, Shotwell SL (1982). Monoclonal antibodies against the *Drosophila* nervous system. *Proc Natl Acad Sci USA* 79, 7929–7933.
- Goldstein LS, Gunawardena S (2000). Flying through the *Drosophila* cytoskeletal genome. *J Cell Biol* 150, F63–F68.
- Goldstein LS, Yang Z (2000). Microtubule-based transport systems in neurons: the roles of kinesins and dyneins. *Annu Rev Neurosci* 23, 39–71.
- Hayashi S, Rubinfeld B, Souza B, Polakis P, Wieschaus E, Levine AJ (1997). A *Drosophila* homolog of the tumor suppressor gene adenomatous polyposis coli down-regulates  $\beta$ -catenin but its zygotic expression is not essential for the regulation of Armadillo. *Proc Natl Acad Sci USA* 94, 242–247.
- Hirokawa N, Takemura R (2005). Molecular motors and mechanisms of directional transport in neurons. *Nat Rev Neurosci* 6, 201–214.
- Hummel T, Schimmelpfeng K, Klämbt C (1997). Fast and efficient egg collection and antibody staining from large numbers of *Drosophila* strains. *Dev Genes Evol* 207, 131–135.
- Hurd DD, Saxton WM (1996). Kinesin mutations cause motor neuron disease phenotypes by disrupting fast axonal transport in *Drosophila*. *Genetics* 144, 1075–1085.
- Lee T, Luo L (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. *Neuron* 22, 451–461.
- Marszalek JR, Weiner JA, Farlow SJ, Chun J, Goldstein LS (1999). Novel dendritic kinesin sorting identified by different process targeting of two related kinesins: KIF21A and KIF21B. *J Cell Biol* 145, 469–479.
- Munemitsu S, Souza B, Muller O, Albert I, Rubinfeld B, Polakis P (1994). The APC gene product associates with microtubules in vivo and promotes their assembly in vitro. *Cancer Res* 54, 3676–3681.
- Saxton WM, Hicks J, Goldstein LS, Raff EC (1991). Kinesin heavy chain is essential for viability and neuromuscular functions in *Drosophila*, but mutants show no defects in mitosis. *Cell* 64, 1093–1102.
- Shah JV, Goldstein LS (2000). Does motor protein intelligence contribute to neuronal polarity? *Neuron* 26, 281–282.
- Shi SH, Cheng T, Jan LY, Jan YN (2004). APC and GSK-3 $\beta$  are involved in mPar3 targeting to the nascent axon and establishment of neuronal polarity. *Curr Biol* 14, 2025–2032.
- Silverman MA, Kaech S, Jareb M, Burack MA, Vogt L, Sonderegger P, Banker G (2001). Sorting and directed transport of membrane proteins during development of hippocampal neurons in culture. *Proc Natl Acad Sci USA* 98, 7051–7057.
- Smith KJ, Levy DB, Maupin P, Pollard TD, Vogelstein B, Kinzler KW (1994). Wild-type but not mutant APC associates with the microtubule cytoskeleton. *Cancer Res* 54, 3672–3675.
- Stone MC, Roegiers F, Rolls MM (2008). Microtubules have opposite orientation in axons and dendrites of *Drosophila* neurons. *Mol Biol Cell* 19, 4112–4119.
- West AE, Neve RL, Buckley KM (1997). Identification of a somatodendritic targeting signal in the cytoplasmic domain of the transferrin receptor. *J Neurosci* 17, 6038–6047.
- Wilson RJ, Goodman JL, Strelets VB (2008). FlyBase: integration and improvements to query tools. *Nucleic Acids Res* 36, D588–D593.
- Zinsmaier KE, Eberle KK, Buchner E, Walter N, Benzer S (1994). Paralysis and early death in cysteine string protein mutants of *Drosophila*. *Science* 263, 977–980.